REMARKS

The claims have been amended to enhance clarity (see claims 39, 41, 53, 59, and 62: these amendments are more fully explained below) and to correct obvious errors that resulted in incorrect claim dependency (claim 77) or indefiniteness due to lack of proper antecedent basis (claims 76 and 78). The amendment to claim 39 also corrects a typographical error in the definition of X^2: it contained "CO" twice, which was obviously an error. The second occurrence of CO was replaced with "SO"; this is supported by the specification at page 5, line 14, which says "X^2 may be any of the alternatives set forth for X^1", combined with the definition for X^1 on the same page (line 7), which includes SO.

The phrase "constituting the substituent R^2" was removed from claims 53 and 59, where it was rendered unnecessary by carriage returns that were inserted to more clearly delineate the phrases defining various substituents. Claims 72, 73 and 74 were amended to correct a typographical error in the name of the first compound (one "i" was missing from "4-benzylpiperidinyl indole-5-carboxamide in each claim). No new matter has been added, and entry of the amendment is respectfully requested.

Indefiniteness Rejections Under 35 U.S.C. § 112, Second Paragraph

The Examiner rejects claim 39, alleging that the phrase "alkyl and aryl optionally including one or more heteroatoms selected from O, S, and N" is indefinite and unclear. Applicants believe that the phrase recited is clear on its face when read in context. The claim reads as follows:

> [E]ach R^4 is independently selected from the group consisting of H, alkyl (1-6C) and aryl, each of said alkyl and aryl optionally including one or more heteroatoms selected from O, S, and N and each of said alkyl being optionally substituted by one or more substituents selected from the group consisting of halo…

The applicants believe that the sentence clearly describes the alkyl and aryl as optionally including one or more heteroatoms in the alkyl or aryl group. The substituents have not been mentioned when the phrase "including one or more heteroatoms" is introduced, thus it would not be

logical for that phrase to refer to those substituents as the Examiner suggests: it could not reasonably refer to anything other than the alkyl or aryl group just mentioned. Furthermore, the substituents subsequently introduced clearly include these heteroatoms. It would be redundant to say "including one or more heteroatoms" and then list the optional substituents themselves if the phrase referred to the presence of these heteroatoms in the optional substituents. Hence, applicants believe that the phrase is clear as it was originally presented.

Nevertheless, the applicants have now amended the claim by inserting 'carriage returns', or 'ends of paragraphs' to emphasize the plain meaning. These are inserted several times to clearly separate phrases; most of the insertions follow a comma that already separated the phrases, but the comma was added where it was missing. The word "and" was also deleted where it was rendered unnecessary. The inserted carriage returns serve only to emphasize the ordinary and intended meaning of the original claim language without modifying its meaning. The applicants believe this amendment clarifies the most logical interpretation of the original language as it would be understood by one of ordinary skill and thus adds no new matter.

Claims 41, 53, 59, and 62 were similarly amended to enhance clarity.

In claim 75, the Examiner objected to the phrase "a condition characterized by a pro-inflammation response," which was alleged to be indefinite. The Examiner asserts that "clinically, proinflammatory response is not inflammation (see Cecil textbook of medicine, disorders of inflammatory response)." The applicant notes that the reference that was provided by the Examiner discusses inflammation, and it does not appear to mention proinflammation; the absence of the term "proinflammation", though, cannot reasonably be interpreted as evidence of what proinflammation does or does not mean. The relevant question is what the term used in the claim means according to the specification, or perhaps what it would mean to one of ordinary skill. The cited reference does not directly address either question; hence it is irrelevant.

The similar term "proinflammatory" is defined in one medical dictionary as "capable of stimulating inflammation." See **Exhibit A**, Dorland's Illustrated Medical Dictionary, 30th ed.

(2003). More importantly, the specification explains the term that is used herein when it discusses the activity of the claimed compounds at page 18, ll. 23-29, for example, where it says, "The compounds of the invention inhibit the production of cytokines such as TNF, IL-1, IL-6, and IL-8, cytokines that are important proinflammatory constituents in many different disease states and syndromes." The applicant believes that this passage describes the same conditions that "characterized by a pro-inflammation response" as used in claim 75 would convey to one of ordinary skill in the art: one of ordinary skill would recognize that a pro-inflammation response refers to a physiological response characterized by the production of proinflammatory cytokines, such as the examples mentioned in the specification.

Proinflammatory cytokines were well-known in the art prior to the filing date as demonstrated by attached **Exhibit B**, which is a published article whose stated objective is "To review the concept of proinflammatory cytokines". "Proinflammatory Cytokines" (see the abstract), C.A. Dinarello, *Chest*, 118(2), 503-508 (Aug. 2000) **[attached as Exhibit B]**. The reference describes proinflammatory cytokines as those which "promote inflammation." The distinguishing characteristic of the conditions within the scope of the claim is the production of proinflammatory cytokines; and standards for recognizing which cytokines are proinflammatory are well known. There is thus a clear standard by which one of ordinary skill could recognize which conditions are within the claim scope and which are not. Therefore, the term is not indefinite: it is explained in the specification, and the closely related term "proinflammatory" has an art-recognized definition that is not inconsistent with the present usage.

The Examiner also alleges that this claim would cover future discovery of such conditions, and uses the term "'reach through' scope" to explain why this is a basis for rejecting the claims. The applicants are confused by this terminology, and assume the examiner is using the term as it was used in some discussions of the recent *University of Rochester* case. See, e.g., Stephen R. Albainy-Jenei and Karlyn A. Schnapp, Early-Stage Companies Face New Challenges; Rochester Case Limited the Patentability Of Reach-Through Claims, 12/8/03 Nat'l L.J. S3, col. 1 (2003) **[Attached as Exhibit C]** and other references summarized in Rader's dissent from the denial of *en banc* rehearing of Univ. of Rochester v. G.D. Searle & Co., 358 F.3d 916 (Fed. Cir. 2004). The

current claim can indeed embrace conditions that have not yet been described if those conditions are characterized by the production of proinflammatory cytokines. Nevertheless, this comports with the Supreme Court's discussion in *Warner-Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17 (1997). In *Warner-Jenkinson*, the Supreme Court acknowledged that a claim embraces not only the specifically recited elements, but also those that are equivalent to the elements recited as judged at the time of infringement, and in describing the reach of this doctrine of equivalence, the Court said:

> Insofar as the question under the doctrine of equivalence is whether an accused element is equivalent to a claimed element, the proper time for evaluating equivalency and thus knowledge of interchangeability between elements is at the time of infringement, not at the time the patent was issued.

This quotation indicates that the Court saw no impropriety in having an earlier patent claim cover later-arising equivalent subject matter. Thus, a claim's scope may encompass after-arising equivalents.

In *Festo*, the Federal Circuit and the Supreme Court both acknowledged that claims should encompass after-arising subject matter that is equivalent to what is literally claimed; in fact, prosecution history estoppel may arise if the applicant fails to claim an embodiment that could have been described. See Festo Corp. v. Shoketsu Kinzoki Kogyo Kabushiki Co., 68 USPQ2d 1321 (Fed. Cir. 2003). But where the patentee could *not* have described it when drafting the application, the after-arising subject matter is within the claim scope if it is deemed equivalent at the time of infringement. Here, the phrase 'characterized by a pro-inflammation response' is believed to collectively and accurately describe those conditions within the scope of the claimed invention, and to indicate what common biochemical / biological origin those conditions share.

Also, the 'after-arising' character of any condition within the described genus would only be the *later recognition of an inherent characteristic* of such conditions: the conditions themselves would not be new. If the Examiner's phrase "reach through scope" refers to the possibility that the claim would encompass conditions discovered at a later time to be *characterized* by the production of proinflammatory cytokines, applicants agree that those are within the scope of

the claims, but believe that such claims are consistent with the interpretations of patent claims by the Federal Circuit and the Supreme Court.

Furthermore, this is not a situation analogous to that in *Rochester*, where the patentee sought to enforce claims to a later *invention*. If the *Rochester* 'reach-through' claims were enforced, the patentee could have prevented an inventor of a later-discovered novel compound from practicing the later invention. This situation is different: if newly *recognized* conditions characterized by production of proinflammatory cytokines are identified, the present claims would only give the applicant the right to prevent others from using the *compounds of the present invention* to treat such conditions. The present claims do *not* allow the applicants to 'reach through' to control a compound later invented by another: they only cover the compounds presently described and certain methods of using these compounds. The use of these compounds to treat conditions characterized by production of proinflammatory cytokines is part of the present invention: the inventors recognize that the invented compounds, which reduce proinflammatory cytokine formation and are thus useful for treating conditions "characterized by a pro-inflammation response", can be used to treat such conditions *regardless of when the character of the conditions is recognized.*

Also, the applicants note that, as a matter of public policy, the danger of so-called 'reach-through' claims as that term is used in discussions of *Rochester* is that they reduce the incentive for others to make later pharmaceutical inventions; the present claims do not have that effect. They are thus not objectionable as 'reach through' claims as far as the applicants understand that term.

Claim 76 is objected to as confusing and lacking antecedent basis in the base claim. Applicants acknowledge that the inflammation and pro-inflammation terms were inadvertently different, and have amended claim 76 to eliminate the phrase "characterized by inflammation." Applicants believe that the remaining phrase, "said condition," finds antecedent basis in claim 75. Likewise, for claims 77 and 78: in claim 78, there was not a specific and clear antecedent basis for the phrase: "said chronic heart condition." That, too, has been corrected by the present amendment,

which removes the word 'chronic' so that claim 78 finds clear antecedent basis in the language of claim 77.

The Rejections Under 35 U.S.C. § 112, First Paragraph, Written Description and Enablement

The Examiner rejects claims 39-71 and 75-77 under 35 U.S.C. § 112, first paragraph, for failure to comply with the written description and enablement requirements. Applicants note that the discussion from that point on appears to relate entirely to enablement. The applicants will address the rejection primarily as though it is based on enablement, since it is expressly based on the *In re Wands* case, which is the classic case for undue experimentation as it relates to enablement. In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). The Examiner discusses three elements of the *Wands* analysis; the response uses the same format for convenience.

"Nature of Invention"

The Examiner asserts that the description of R^4 lacks sufficient descriptive and enabling support in the specification, and then says, "No explicit description can be found as to what the intended chemical structure is for such terms." Applicants believe, as discussed above, that R^4 is fully described by the language that was provided in the claims as originally written: one of ordinary skill, reading the claim as drafted and reading the same language in the specification, would understand exactly which chemical structures are and are not embraced by the scope of the claim. The amended version of the claims is even clearer about what claim limitations apply to which features of R^4. Hence the applicants believe that the chemical structure of R^4 is adequately and explicitly described to one of ordinary skill in the art by the language of the claim, and no written description deficiency exists.

As to enablement, the specification teaches the preparation of compounds wherein R^4 is an alkyl group substituted by an amine (pg. 17) or cyclic amine (pg. 57); dicarbonyl groups containing acid and amide groups (e.g., pg. 17), including cyclic amides and amine-substituted alkyl amides (e.g., pg. 59); H (throughout, including, e.g., pg. 39); substituted amides ((pg. 42); trifluoroacetyl (pg. 53); carboxylic acid (pg. 53); and a diverse set of additional amides (pg. 56)

containg amine, alkoxy, arylalkyl, and substituted aryl groups. It thus describes and enables compounds having a wide array of R^4 groups.

"The State of the Art and Predictability"

The Examiner cites two references: CA 139: 117268 (CA 139) and CA 131: 67650 (CA 131), each of which relates to compound families that are arguably structurally similar to the claimed genus, and each of which recites a different biological activity for its own chemical genus. The Examiner observes that these two different genera exhibit different activities from the present genus despite some similarities in structure, and then says, "the drastic diversity in utility resulted [sic] from small chemical structure [sic: structure modifications?] all fall within the claimed scope indicated the high degree of unpredictability of such compounds." The applicants interpret this statement to suggest that since a structurally similar genus in each reference has different biological or biochemical activity from that of the present genus, this particular area of chemistry is somehow shown to be "unpredictable."

The applicants point out, with all due respect, that it is well known to those of ordinary skill that a *single compound* can have multiple biochemical or biological activities: aspirin is perhaps the best known example. Aspirin has analgesic, antipyretic and anticoagulant activities, and is known to inhibit both cyclooxygenases COX-1 and COX-2 (see, for example, the discussion in University of Rochester v. G.D. Searle & Co., 69 USPQ2d 1886 (Fed. Cir. 2004)); it is also known to strongly inhibit other enzymes, including IKK-beta (M.J. Yin, et al., *The antiinflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta*, Nature, 396(6706), 5 Nov 1998, 77-80) **[Attached as Exhibit D]**. Indeed, *many* drugs have side effects caused by interaction of the drug with other biochemical processes besides the intended one. See, e.g., *Foye's Principles of Medicinal Chemistry*, pg. 56-57 (5th ed., 2002) **[Attached as Exhibit E]**, discussing the evolution of a family of antihistamines into sedatives and antipshychotics based on observations of the side effects that each drug caused in treated patients; or see the latest news on Vioxx™ litigation.) Thus it would not be at all surprising for the members of *any* genus of compounds to have multiple biological or biochemical effects. The presence of other biochemical or biological activities in

structurally related genera does not lead one of ordinary skill to doubt that the compounds of the genus defined in the present claims *lack* the activity that is asserted in the specification, though; at most one could infer that the several genera may also have overlapping activity in addition to that which is disclosed for each separate genus. But the possibility that the claimed compounds exhibit other activities *in addition* to those asserted does not cause one to reasonably question that the claimed genus possesses the asserted utility.

The Examiner seems to believe that, because CA 139 recites treating proliferative disease and CA 131 recites thrombin inhibition activity, the compounds in those families must each have ONLY the activity ascribed to them. One of ordinary skill in the art, however, would understand that the activities of the two families of compounds could well overlap, and that compounds of the present invention could exhibit the same kinds of activity described for the compound genera in CA 131 or CA 139 or both. Thus, it is unclear to applicants why the compounds in the two cited references have *any* impact on the predictability or unpredictability of activity within the family of the claimed compounds. Whether the claimed compounds possess activity analogous to the activities of the compounds described in CA 139 and CA 131 is not relevant to the credibility of the assertion that they possess the activity presently asserted: it raises an unrelated question (i.e., what *other* activities might the claimed compounds possess?), but it does not create reasonable doubt that the claimed compounds have the activity asserted. Therefore, it is not relevant to the patentability of the present claims.

The Amount of Guidance and Working Examples

Applicants note that the Examiner specifically singles out compounds where X^1 is a sulfonyl (SO_2), and says that none have been tested or reported to have p38 kinase activity. Applicants are confused by this, because the Office Action states that the Examiner has entered the claim set offered in the final amendment dated February 27, 2004. In that claim set, SO_2 was deleted from the definition of X^1; the claims currently presented reflect that earlier amendment. Thus, it is unclear why the sulfonyl is identified as an element of an enablement rejection.

The Examiner next states that "none of the compounds wherein R^4 is broadly aryl" have been made or tested. Applicants point to compounds identified as iv, v, vi and vii on page 56, each of which contains an aryl ring on a substituted alkyl linker (arylalkyl groups). Based on these compounds, one of ordinary skill would expect compounds where R^4 is an aryl group *directly* linked to the central ring, to have similar activity: the arylalkyl group can occupy much of the same binding site volume and may have similar binding characteristics to an aryl. And based on the large number of active compounds disclosed with alkyl, substituted alkyl, arylalkyl and acyl groups for R^4, applicants believe that a scope including aryl as one potential R^4 group is adequately supported by the specification.

Applicants find the next paragraph in the Office Action confusing but will attempt to fully respond to it. The paragraph says, "In view of the diversity of utility based on the bicyclic core with distinct substitution as evidenced *supra*, the lacking of variation for the Markush scope with such breadth finds this claimed scope lacks description as well as enablement."

Applicants assert that the apparently diverse utilities are united by the common biochemical and biological pathways that they derive from. All of the utilities are based on the inhibition of the production of proinflammatory cytokines, see specification, pg. 18 ll. 23-26: "The compounds of the invention inhibit the production of cytokines such as TNF, IL-1, IL-6, and IL-8, cytokines that are important proinflammatory constituents in many different disease states and syndromes. Thus, inhibition of these cytokines has benefit in controlling and mitigating many disease states and syndromes." Hence, the "diversity of utility" is more a matter of perception than reality. The utility for these compounds appears broad because they inhibit a biochemical step (proinflammatory cytokine formation) that has many external manifestations. The biological results produced appear to be diverse, but all the results are produced by a common biochemical activity: inhibition of formation of proinflammatory cytokines. Thus inhibiting the production of these cytokines by using p38 inhibitors of the present invention may have multiple medical uses. Nevertheless, the utility itself only seems diverse because the biochemical event that the compounds of the invention control manifests itself in the form of various seemingly diverse medical conditions.

The applicants have already addressed specific questions regarding the scope of the chemical genus claimed, and are unsure what else may be meant by the Examiner's phrase "lacking of variation for the Markush scope". Thus applicants have responded as well as they are able from the wording of the rejection by addressing both the scope of asserted utility and the scope of the compound genus.

Furthermore, the applicants point out that the *Wands* factors provide a balancing test, and must be considered as a whole. The court in *Wands* provided eight factors to consider: (1) quantity of experimentation necessary (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. The above arguments address the Examiner's analysis of a few of these, but a *Wands* analysis should also consider the following points.

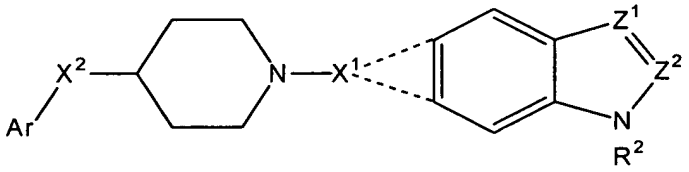
Amount of direction or guidance presented. The specification includes five tables (Tables 1-5) of data for specific compounds, and page 66 provides data for several more. Claim 72 lists 35 specific compounds described in the specification in addition to the compounds of Table 5. Thus the specification provides extensive guidance on the selection of compounds for use in treating conditions characterized by excessive production of cytokines, and the determination of a compound's biochemical effect is a routine matter as is determination of an effective dose.

Presence of working examples. The specification is replete with working examples of the making and biochemical testing of the claimed compounds, and with other guidance on practicing the claimed invention. The applicants also remind the Examiner that the key word in the *Wands* analysis is not 'experimentation', it is 'undue.' In re Angstadt, 537 F.2d 498, 190 USPQ 214 (CCPA 1976). Thus the necessity for routine experimentation is not inappropriate.

As to the Examiner's statement that the "claimed scope lacks description as well as enablement,", the applicants point out that Federal Circuit case law indicates that a description by structure of a compound or genus of compounds satisfies the written description requirement. See

Regents of the Univ. of Calif. v. Eli Lilly & Co., 119 F.3d 1559 (Fed. Cir. 1997), (saying that a written description of DNA "requires a precise definition, such as by structure, formula, chemical name or physical properties".) Only where there is insufficient structural information for one of ordinary skill to recognize that the applicant "invented the claimed invention" is there a written description issue. See *Eli Lilly*. Here, in addition to a genus that is well-defined by structure, the applicants provide a large number of exemplary compounds: claim 72 names about 35 different compounds within the claim scope. The applicants have addressed the allegation that the language in the description of substituents in the genus description was indefinite, and the applicants do not understand what else the Examiner would base a 'written description' rejection on in this case.

Breadth of the claims. In fact, the claimed compound genus is fairly well defined. It comprises a central bicyclic aromatic ring that is limited to indole, benzimidazole, benzotriazole, or benzopyrazole; to this ring, a piperidine ring is attached. The piperidine is connected to the bicyclic group specifically through the piperidine nitrogen, is separated from the bicyclic group by one atom of the linking group X^1, and X^1 is clearly and narrowly defined. X^1 can only be attached to the central ring at one of the two positions indicated by the dashed lines in the figure below. At C-4 of the piperidine ring, a substituent X^2-Ar is attached, and X^2 is defined narrowly so that precisely one atom separates the piperidine ring from the group Ar. Ar is also narrowly defined to be a phenyl ring or two phenyl rings. While other substituents may be added at several positions, these limitations define a reasonably narrow genus of compounds that the applicants believe is well supported by the numerous examples provided.



As mentioned above, *Wands* provides a balancing test with eight factors. The applicants have rebutted the Examiner's contention that three of those factors weigh against finding that the specification provides adequate enablement for the claim scope sought. The applicants have also demonstrated that several of the remaining factors operate in favor of finding adequate enablement.

Even if the Examiner disagrees with some of the preceding arguments, the applicants have thus shown that, on balance, the specification adequately describes and enables the claims. Withdrawal of these rejections is thus requested.

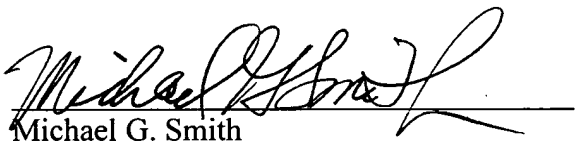
The Examiner also notes that if the treated conditions within the scope of the claims include arthritis, a 102(f) or (g) issue may have to be resolved with the CA 139 reference. In response, applicants point out that the filing date for the current application is February 13, 2002. It is a divisional of US Application No. 09/316,761 which was filed 21 May 1999. The present application thus has a priority date not later than 21 May 1999. The CA 139 reference, which only claims a priority date of January 7, 2002 is not prior art to this application.

CONCLUSION

The applicants believe that the preceding amendments and arguments address each ground for rejection imposed by the Examiner and respectfully request reconsideration. In the event that the Examiner finds that some of the claim language presented could be modified for better clarity, the applicants would welcome suggestions from the Examiner.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 219002028310.

Respectfully submitted,

Dated: January 26, 2005 By: 

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The Curtis Center
Independence Square West
Philadelphia, PA 19106

Dorland's illustrated medical dictionary.
Philadelphia: Saunders

v.: ill.; 27 cm.

Irregular.
Began publication with 23rd ed.
Description based on: 26th ed.
Continues: American illustrated medical dictionary.

1. Medicine—Dictionaries. I. Dorland, W.A. Newman (William Alexander Newman), 1864–1956.
[DNLM: 1. Dictionaries, Medical. 2. Reference Books, Medical]

R121.D73 610'.3'21—dc19 0-6383
AACR 2 MARC-S

Library of Congress [8607r85]rev6

Chief Lexicographer: Douglas M. Anderson, MA
Senior Lexicographer: Patricia D. Novak, PhD
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Portuguese (*28th Edition*)—Editiora Manole Ltda., São Paulo, Brazil

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Dorland's Illustrated Medical Dictionary

ISBN 0-7216-0146-4 (Standard)
0-7216-0281-9 (Deluxe)
0-7216-0414-5 (Trade)
0-8089-2288-2 (International)

Library of Congress catalog card number 78-50050

Last digit is the print number: 9 8 7 6 5 4 3 2 1

■ Mandibular prognathism.

projecting jaws; having a gnathic index above 103, the teeth being in mesioclusion. Called also *prognathic*.

prog·nose (prog-nōs′) prognosticate.

prog·no·sis (prog-no′sis) [Gr. *prognōsis* foreknowledge] a forecast as to the probable outcome of an attack of disease; the prospect as to recovery from a disease as indicated by the nature and symptoms of the case.

prog·nos·tic (prog-nos′tik) 1. affording an indication as to prognosis. 2. a symptom or sign on which a prognosis may be based.

prog·nos·ti·cate (prog-nos′ti-kāt) to forecast the probable outcome of an attack of disease.

prog·nos·ti·cian (prog″nos-tish′ən) one who is skilled in prognosis.

pro·go·no·ma (pro″go-no′mə) [*pro-* + *gon-* + *-oma*] a tumor due to misplacement of tissue as the result of fetal atavism to a stage which does not occur in the life history of the species, but which does occur in ancestral forms of the species.

melanotic p. melanotic neuroectodermal tumor.

Pro·graf (pro′graf) trademark for preparations of tacrolimus.

pro·gram·ming (pro′gram″ing) the provision of an ordered set of instructions or procedures.

neurolinguistic p. a complementary therapeutic strategy based on the premise that thought is a representation of sensory experience and that behavior can be modified to achieve a desired result by changing the patient's thought patterns and mental strategies in order to give the patient more choices in problem-solving; used for behavior modification and the management of psychosomatic disorders and stress.

pro·gran·u·lo·cyte (pro-gran′u-lo-sīt″) promyelocyte.

pro·grav·id (pro-grav′id) [*pro-* + *gravid*] denoting the phase of the endometrium, under the influence of the corpus luteum, during which it is prepared for pregnancy.

pro·gres·sion (prə-gresh′ən) 1. the act of moving or walking forward; see also *gait*. 2. the process of spreading or becoming more severe.

backward p. retropulsion.

cross-legged p. scissors gait.

saltatory p. saltation (def. 5).

pro·gres·sive (pro-gres′iv) advancing; going forward; going from bad to worse; increasing in scope or severity.

pro·guan·il hy·dro·chlo·ride (pro-gwahn′əl) an antimalarial used in the prophylaxis and treatment of malaria, administered orally. Seldom used in the United States because of the development of resistance by the malarial parasite to proguanil. Called also *chloroguanide hydrochloride*.

Pro·HIB·iT (pro-hib′it) trademark for a preparation of *Haemophilus* b conjugate vaccine.

pro·hor·mone (pro-hor′mōn) any substance that can be converted into a hormone; see also *prehormone*. Called also hormonogen.

pro·in·flam·ma·to·ry capable of stimulating inflammation.

pro·in·su·lin (pro-in′su-lin) a precursor of insulin, with a molecular weight of 8,000 to 10,000; it has minimal hormonal activity and is converted to insulin by removal of the connecting C peptide, leaving the two (A and B)-chain, active insulin molecule.

pro·jec·tion (pro-jek′shən) [*pro-* + L. *jacēre* to throw] 1. a throwing forward, especially the act of referring impressions made on the sense organs to their proper source, so as to locate correctly the objects producing them. 2. the connection between the cerebral cortex and other parts of the nervous system or organs of special sense. 3. the act of extending or jutting out, or a part that juts out. 4. in psychiatry, an unconscious defense mechanism in which a person attributes to someone else unacknowledged ideas, thoughts, feelings, and impulses that he finds undesirable or unacceptable in himself. 5. the orientation of a radiographic machine in relation to the body or a body part; called also *view*.

anteroposterior (AP) p. a radiographic projection in which the central ray goes from the front to the back of the body or part, with the film at the back.

axial p. a radiographic projection in which the central ray goes from the base to the vertex or from the vertex to the base of a structure.

axillary p. a radiographic projection in which the patient is supine, the upper limb is abducted, and the central ray enters the axilla at an angle; used for visualizing structures in the shoulder region.

brow-down p. a posteroanterior projection of the head with the patient prone.

brow-up p. an anteroposterior projection of the head with the patient supine.

Caldwell's p. a posteroanterior projection of the head, used for viewing the frontal and anterior ethmoidal sinuses; the central ray enters the back of the head from a slightly superior angle.

carpal tunnel p. a radiographic projection with the wrist hyperextended and the central ray entering the proximal palm at an angle, for visualization of bones and other structures of the proximal palm and wrist.

cross-table p. a radiographic projection of the spine, pelvis, or lower limb with the patient either prone or supine on a table and the central ray entering laterally.

Didiée's p. a rare type of radiographic projection for evaluation of an unstable or repeatedly dislocating shoulder that may have a subtle condition such as the Hill-Sachs lesion; the patient lies prone and the central ray enters the shoulder from a lateral oblique direction.

dorsoplantar p. a radiographic projection of the foot with the central ray passing from the dorsal surface to the plantar surface.

eccentric p. referred sensation.

erroneous p., false p. a misjudging of the position of an object, due to weakness or paralysis of the eye muscles.

frog-leg p. an anteroposterior projection of the abducted hips.

frontal p. a radiographic projection in which the central ray is perpendicular to the frontal plane; it may be either anteroposterior or posteroanterior.

half-axial p. a radiographic projection of the head with the central ray at an angle to the frontal and medial planes; it may be either anteroposterior or posteroanterior. Called also *semiaxial p.*

Heinig's p. a radiographic projection for visualization of the sternum and sternoclavicular joint; the upper limb closer to the tube is abducted over the head and the central ray enters the body from an inferior lateral direction, angling towards the opposite shoulder.

Hermodsson's p. a rare type of radiographic projection for evaluation of an unstable or repeatedly dislocating shoulder that may have a subtle condition such as the Hill-Sachs lesion; the patient stands with the affected upper limb behind the back and the hand over the lumbar vertebrae, and the central ray enters laterally to the scapula from an inferior posterior direction.

Hughston's p. a radiographic projection of the patellofemoral region, with the patient prone, lower limbs at 50 to 60 degrees of flexion, and the central ray at 45 degrees from vertical passing tangentially across the knee.

inferosuperior p. any radiographic projection in which the central ray enters the body or a body part from below.

lateral p. a radiographic projection in which the central ray enters the body or part from the side and is perpendicular to the medial or axial plane.

Laurin's p. an axial radiograph of the knees made with the patient seated with the knees flexed at 30 degrees and the lower limbs together; the x-ray tube is placed between the patient's feet and the film is placed against the anterior thighs perpendicular to the beam.

Merchant's p. an axial radiograph of the knees made with the patient supine, knees flexed at 45 degrees over the table end, and lower limbs together; the tube is angled 30 degrees below the horizontal and the film is held on the tibia perpendicular to the beam.

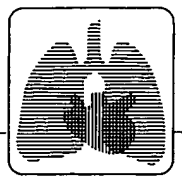
mortise p. an anteroposterior projection of the ankle with the foot rotated internally 15 to 20 degrees so that the bases of the tibia and fibula are no longer in front of the talus.

notch p. 1. Stryker's notch p. 2. a radiographic projection for visualization of the intercondylar notch of the femur.

oblique p. a radiographic projection in which the central ray enters the body or part at an angle to the frontal and medial or axial planes.

open-mouth p. an anteroposterior projection of the cervical spine, particularly the axis and atlas, done through the open mouth with the patient supine and the central ray pointing vertically downward.

pillar p. a radiographic projection of the articular pillars of the cervical spine, usually with the patient supine and the central ray angled slightly towards the feet from a position above the face; for an unobstructed view, the head may be rotated so that the mandible is not above the spine.



impact of basic research on tomorrow's medicine

Proinflammatory Cytokines*

Charles A. Dinarello, MD

Study objectives: **To review the concept of proinflammatory cytokines.**
Design: **Review of published literature.**
Setting: **Academic (university hospital).**
Results: **Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. Some cytokines act to make disease worse (proinflammatory), whereas others serve to reduce inflammation and promote healing (anti-inflammatory). Attention also has focused on blocking cytokines, which are harmful to the host, particularly during overwhelming infection. Interleukin (IL)-1 and tumor necrosis factor (TNF) are proinflammatory cytokines, and when they are administered to humans, they produce fever, inflammation, tissue destruction, and, in some cases, shock and death. Reducing the biological activities of IL-1 and TNF is accomplished by several different, but highly specific, strategies, which involve neutralizing antibodies, soluble receptors, receptor antagonist, and inhibitors of proteases that convert inactive precursors to active, mature molecules. Blocking IL-1 or TNF has been highly successful in patients with rheumatoid arthritis, inflammatory bowel disease, or graft-vs-host disease but distinctly has not been successful in humans with sepsis. Agents such as TNF-neutralizing antibodies, soluble TNF receptors, and IL-1 receptor antagonist have been infused into > 10,000 patients in double-blind, placebo-controlled trials. Although there has been a highly consistent small increase (2 to 3%) in 28-day survival rates with anticytokine therapy, the effect has not been statistically significant.**
Conclusions: **Anticytokine therapy should be able to "rescue" the patient whose condition continues to deteriorate in the face of considerable support efforts. Unfortunately, it remains difficult to identify those patients who would benefit from anticytokine therapy for septic shock.**
(CHEST 2000; 118:503–508)

Key words: chemokines; infection; inflammation; interferon; interleukin-1; sepsis; tumor necrosis factor

Abbreviations: AP = activating protein; COX = cyclooxygenase; IFN = interferon; IL = interleukin; IL-1R = interleukin-1 receptor; IL-1RI = type I interleukin-1 receptor; IL-1Ra = interleukin-1 receptor antagonist; IL-1RII = type II interleukin-1 receptor; IL-1R-AcP = interleukin-1 receptor accessory protein; MAPK = mitogen-activated protein kinase; NF = nuclear factor; NO = nitric oxide; PG = prostaglandin; PL = phospholipase; RA = receptor antagonist; TGF = transforming growth factor; TLR = Toll-like receptor; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor; TRADD = tumor necrosis factor receptor-associated death domain

Cytokines are small, nonstructural proteins with molecular weights ranging from 8 to 40,000 d. Originally called lymphokines and monokines to indicate their cellular sources, it became clear that the term "cytokine" is the best description, since nearly all nucleated cells are capable of synthesizing these proteins and, in turn, of responding to them. There is no amino acid sequence motif or three-dimensional structure that links cytokines; rather, their biological activities allow us to group them into different classes. For the most part, cytokines are primarily involved in host responses to disease or infection, and any involvement with homeostatic mechanisms has been less than dramatic.

Many scientists have made the analogy of cytokines to hormones, but, on closer examination, this is not an accurate comparison. Why? First, hormones tend to be constitutively expressed by highly specialized tissues, but cytokines are synthesized by nearly every cell. Whereas hormones are the primary synthetic product

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Supported by National Institutes of Health Grant AI-15614.
Manuscript received April 6, 2000; revision accepted April 11, 2000.
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of a cell (insulin, thyroid, adrenocorticotropic hormone, etc), cytokines account for a rather small amount of the synthetic output of a cell. In addition, hormones are expressed in response to homeostatic control signals, many of which are part of a daily cycle. In contrast, most cytokine genes are not expressed (at least at the translational level) unless specifically stimulated by noxious events. In fact, it has become clear that the triggering of cytokine gene expression is nearly identical to "cell stressors." For example, ultraviolet light, heat-shock, hyperosmolarity, or adherence to a foreign surface activate the mitogen-activated protein kinases (MAPKs), which phosphorylate transcription factors for gene expression. Of course, infection and inflammatory products also use the MAPK pathway for initiating cytokine gene expression. One concludes then that cytokines themselves are produced in response to "stress," whereas most hormones are produced by a daily intrinsic clock.

Cytokine Responses to Infection and Inflammation

There are presently 18 cytokines with the name *interleukin* (IL). Other cytokines have retained their original biological description, such as *tumor necrosis factor* (TNF). Another way to look at some cytokines is their role in infection and/or inflammation. Some cytokines clearly promote inflammation and are called *proinflammatory cytokines*, whereas other cytokines suppress the activity of proinflammatory cytokines and are called *anti-inflammatory cytokines*. For example, IL-4, IL-10, and IL-13 are potent activators of B lymphocytes. However, IL-4, IL-10, and IL-13 are also potent anti-inflammatory agents. They are anti-inflammatory cytokines by virtue of their ability to suppress genes for proinflammatory cytokines such as IL-1, TNF, and the chemokines.

Interferon (IFN)-γ is another example of the pleiotropic nature of cytokines. Like IFN-α and IFN-β, IFN-γ possesses antiviral activity. IFN-γ is also an activator of the pathway that leads to cytotoxic T cells. However, IFN-γ is considered a proinflammatory cytokine because it augments TNF activity and induces nitric oxide (NO). Therefore, listing cytokines in various categories should be done with an open mind, in that, depending on the biological process, any cytokine may function differentially.

The Concept of Proinflammatory and Anti-inflammatory Cytokines

The concept that some cytokines function primarily to induce inflammation while others suppress inflammation is fundamental to cytokine biology and also to clinical medicine. The concept is based on the genes coding for the synthesis of small mediator molecules that are up-regulated during inflammation. For example, genes that are proinflammatory are type II phospholipase (PL) A_2, cyclooxygenase (COX)-2, and inducible NO synthase. These genes code for enzymes that increase the synthesis of platelet-activating factor and leukotrienes, prostanoids, and NO. Another class of genes that are proinflammatory are chemokines, which are small peptides (8,000 d) that facilitate the passage of leukocytes from the circulation into the tissues. The prototypical chemokine is the neutrophil chemoattractant IL-8. IL-8 also activates neutrophils to degranulate and cause tissue damage. IL-1 and TNF are inducers of endothelial adhesion molecules, which are essential for the adhesion of leukocytes to the endothelial surface prior to emigration into the tissues. Taken together, proinflammatory cytokine-mediated inflammation is a cascade of gene products usually not produced in healthy persons. What triggers the expression of these genes? Although inflammatory products such as endotoxins trigger it, the cytokines IL-1 and TNF (and in some cases IFN-γ) are particularly effective in stimulating the expression of these genes. Moreover, IL-1 and TNF act synergistically in this process. Whether induced by an infection, trauma, ischemia, immune-activated T cells, or toxins, IL-1 and TNF initiate the cascade of inflammatory mediators by targeting the endothelium. Figure 1 illustrates the inflammatory cascade triggered by IL-1 and TNF.

Anti-inflammatory cytokines block this process or at least suppress the intensity of the cascade. Cytokines such as IL-4, IL-10, IL-13, and transforming growth factor (TGF)-β suppress the production of IL-1, TNF, chemokines such as IL-8, and vascular adhesion molecules. Therefore, a "balance" between the effects of proinflammatory and anti-inflammatory cytokines is thought to determine the outcome of disease, whether in the short term or long term. In fact, some studies have data suggesting that susceptibility to disease is genetically determined by the balance or expression of either proinflammatory or anti-inflammatory cytokines. However, gene linkage studies are often difficult to interpret. Nevertheless, the deletion of the IL-10 gene in mice results in the spontaneous development of a fatal inflammatory bowel disease. Deletion of the TGF-β1 gene also results in a spontaneous inflammatory disease. In mice deficient in IL-1 receptor antagonist (IL-Ra), spontaneous disease that is nearly identical to rheumatoid arthritis is observed.

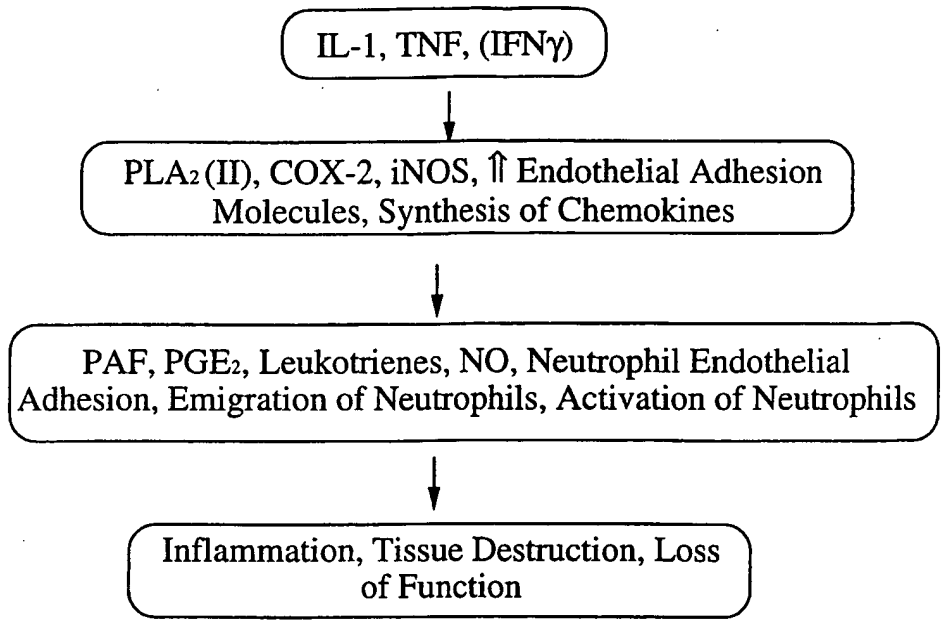

FIGURE 1. The inflammatory cascade triggered by IL-1 and TNF. iNOS = inducible NO synthase; PAF = platelet-activating factor.

IL-1 AND TNF

The synergism of IL-1 and TNF is a commonly reported phenomenon. Clearly, both cytokines are being produced at sites of local inflammation, and, hence, the net effect should be considered when making correlations between cytokine levels and severity of disease. There is also synergism between IL-1 and bradykinin as well as between IL-1 or TNF and mesenchymal growth factors. Most relevant to pain is the increase in prostaglandin (PG)-E_2 stimulated by IL-1 or the combination of IL-1 and TNF. IL-1 also lowers the threshold of pain primarily by increasing PGE_2 synthesis.[1] Table 1 summarizes the synergism of IL-1 and TNF.

Humans injected with IL-1 experience fever, headache, myalgias, and arthralgias, each of which is reduced by the coadministration of COX inhibitors.[2] One of the more universal activities of IL-1 is the induction of gene expression for type II PLA_2 and COX-2. IL-1 induces the transcription of COX-2 and seems to have little effect on the increased production of COX-1. Moreover, once triggered, COX-2 production is elevated for several hours and large amounts of PGE_2 are produced in cells stimulated with IL-1. Therefore, it comes as no surprise that many biological activities of IL-1 are actually due to increased PGE_2 production. There appears to be selectivity in COX inhibitors, in that some nonsteroidal anti-inflammatory agents are better inhibitors of COX-2 than of COX-1. Similar to COX-2 induction, IL-1 preferentially stimulates new transcripts for the inducible type II form of PLA_2, which cleaves the fatty acid in the number 2 position of cell membrane phospholipids. In most cases, this is arachidonic acid. The release of arachidonic acid is the rate-limiting step in the synthesis of PGs and leukotrienes. IL-1 also stimulates increased leukotriene synthesis in many cells.

IL-1 RECEPTORS AND SIGNAL TRANSDUCTION

Receptors

Two primary IL-1 binding proteins (IL-1 receptors [IL-1Rs]) and one IL-1R accessory protein (IL-1R-AcP) have been identified.[3,4] The extracellular domains of the two IL-1Rs and the IL-1R-AcP are members of the Ig superfamily, are each composed of three IgG-like domains, and share a significant homology to each other. The two IL-1Rs are distinct gene products, and, in humans, the genes for type I IL-1R (IL-1RI) and type II IL-1R (IL-1RII) are located on the long arm of chromosome 2.[5]

In primary cells, there are < 50 IL-1Rs per cell, and IL-1 signal transduction has been observed in cells expressing < 10 IL-1RIs per cell. Interestingly, the cytosolic domain of IL-1RI has a 45% amino acid homology with the cytosolic domain of the Drosophila *Toll* gene.[6] Toll is a transmembrane protein that acts like a receptor. There are several mammalian receptors called Toll-like receptors (TLRs). The

Table 1—*Synergistic Activities of IL-1 and TNF

Cytokines	Activities
IL-1 plus TNF	Hemodynamic shock and lactic acidosis in rabbits
	Radioprotection
	Generation of Shwartzman reaction
	Luteal cell $PGF_{2\alpha}$ synthesis
	PGE_2 synthesis in fibroblasts
	Galactosamine-induced hepatotoxicity
	Sickness behavior in mice
	Circulating NO and hypoglycemia in malaria
	Nerve growth factor synthesis from fibroblasts
	Insulin release and β islet cell death
	Insulin resistance
	Loss of lean body mass
	IL-8 synthesis by mesothelial cells
IL-1 plus bradykinin	Angiogenesis
	PGE_2 synthesis in gingival fibroblasts
	Arachidonic acid release from synoviocytes
	$PGF_{2\alpha}$ synthesis in uterine decidua
	IL-6 production from hepatoma cells and fibroblasts
IL-1 or TNF plus FGF or PDGF or EGF or TGF-α	PGE_2 synthesis in dermal fibroblasts
	PGE_2 synthesis in synovial cells
	Chemotaxis for fibroblasts
	PLA_2 release from synoviocytes
	Degradation of articular cartilage
	PGE_2 synthesis in osteoblastic cells

*FGF = fibroblast growth factor; EGF = epidermal growth factor; PDGF = platelet-derived growth factor.

ligands for two of these are endotoxin (TLR-4)[7] and peptidoglycan (TLR-2). The cytoplasmic domains of the TLR are nearly identical to those of IL-1 and IL-18.[8] Gene organization and amino acid homology suggest that the IL-1RI and the cytosolic Toll are derived from a common ancestor (Toll) and trigger similar signals.[9]

Like other models of two-chain receptors, IL-1 binds first to the IL-1RI with a low affinity. The crystal structure of the IL-1RI complexed with IL-1β has been reported and sheds light on the changes that take place after the low-affinity binding.[10] The two receptor-binding sites of IL-1β have been reported using specific mutations. The crystal structure reveals that both receptor-binding sites contact the IL-1RI at the first and third domains.[10] On contact with the first domain, there appears to be a change in the rigidity of the third domain to encounter contact with the second binding site of IL-1β. IL-1β itself does not undergo a structural change. IL-1Ra has only one binding site,[11] and its absence prevents contact with the third domain. Hence, the critical contact point appears to be at the third domain. Since this contact is likely to be absent in complexes with the IL-1Ra,[12] the structural change in the IL-1RI third domain may allow docking of the IL-1R-AcP with the IL-1RI/IL-1β complex. Without the complex of IL-1R-AcP/IL-1RI/IL-1β, there is no signal transduction.[4]

Antibodies to IL-1RI and IL-1R-AcP block IL-1 binding and activity.[4] IL-1R-AcP is essential to IL-1 signaling; in cells deficient in IL-1R-AcP, no IL-1-induced activation of the stress kinases takes place, but this response is restored on transfection with a construct expressing IL-1R-AcP.[13] Affinity-purified antibodies to the IL-1R-AcP third domain amino acids preferentially block IL-1β activity,[14] suggesting that the docking of IL-1R-AcP with IL-1RI takes place within the third domain of each receptor.

IL-1 Decoy Receptor

IL-1RII has a short cytosolic domain consisting of 29 amino acids. IL-1RII appears to act as "decoy" molecule, particularly for IL-1β. The receptor binds IL-β tightly, thus preventing binding to the signal-transducing IL-1RI.[15] It is the lack of a signal-transducing cytosolic domain that makes IL-1RII a functionally negative receptor.

Signal Transduction

Within a few minutes following binding to cells, IL-1 induces several biochemical events.[16–19] It remains unclear which is the most "upstream" triggering event or whether several events occur at the same time. No sequential order or cascade has been identified, but several signaling events appear to be taking place during the first 2 to 5 min. Some of the biochemical changes associated with signal transduction are likely to be cell-specific. In general, multiple protein phosphorylations and activation of phosphatases can be observed within 5 min,[20] and some are thought to be initiated by the release of lipid mediators. The release of ceramide has attracted attention as a possible early signaling event.[21] Phosphorylation of PLA_2 activating protein (AP) also occurs in the first few minutes,[22] which leads to a rapid release of arachidonic acid. Multiple and similar signaling events also have been reported for TNF.

With few exceptions, there is general agreement that IL-1 does not stimulate either hydrolysis of phosphatidylinositol or an increase in intracellular calcium. Without a clear increase in intracellular calcium, early postreceptor binding events nevertheless include hydrolysis of a guanosine 5′-triphosphate with no associated increase in adenyl cyclase, activation of adenyl cyclase,[23,24] hydrolysis of phospholipids,[25,26] release of ceramide,[27] and release of arachidonic acid from phospholipids via cytosolic PLA_2 following its activation by PLA_2 AP.[22] Some IL-1

signaling events are prominent in different cells. Postreceptor signaling mechanisms may, therefore, provide cellular specificity. For example, in some cells, IL-1 is a growth factor, and signaling is associated with serine/threonine phosphorylation of the MAPK p42/44 in mesangial cells.[28] The MAPK p38, another member of the MAPK family, is phosphorylated in fibroblasts,[29] as is the p54α MAPK in hepatocytes.[30]

IL-1-induces several transcription factors. Most of the biological effects of IL-1 take place in cells following nuclear translocation of nuclear factor (NF)-κB and AP-1, two NFs that are common to many IL-1-induced genes. In T lymphocytes and cultured hepatocytes, the addition of IL-1 increases the nuclear binding of *c-jun* and *c-fos*, the two components of AP-1.[31] Similar to those for NF-κB, AP-1 sites are present in the promoter regions of many IL-1-inducible genes. IL-1 also increases the transcription of *c-jun* by activating two novel NFs (jun-1 and jun-2) that bind to the promoter of the *c-jun* gene and stimulate *c-jun* transcription.[32]

How Does IL-1 Differ From TNF in Activating Cells?

From the above descriptions of IL-1R and IL-1 signal transduction, we can see that many of these pathways are shared with TNF. Although the receptors for TNF and IL-1 are clearly different, the postreceptor events are amazingly similar. Thus, the finding that IL-1 and TNF activate the same portfolio of genes is not surprising. However, given the same cell and given the same array of activated genes, IL-1 does not result in programmed cell death, whereas TNF does. This can be seen in TNF-responsive fibroblasts in which IL-1 and TNF induce IL-8 but in the presence of actinomycin or cycloheximide, but in which TNF induces classic apoptosis but IL-1 does not. IL-1 will often synergize with TNF for NO induction, and, under those conditions, NO mediates cell death. The best example of this can be found in the insulin-producing β cells in the islets of Langerhans in the pancreas.[33] Unlike IL-1, the receptors for TNF are homodimers and trimers, and, hence, the recruitment of kinases is somewhat different. However, the cytosolic domain of the TNF p55 receptor contains a "death domain" that recruits intracellular molecules involved with initiating programmed cell death.[34] There is no comparable death domain in the cytoplasmic domains of either IL-1RI or IL-1R-AcP.

There are two receptors for TNF, the p55 receptor and the p75 receptor.[35] Although TNF binds and triggers both receptors, the cytosolic domains of these receptors recruit different proteins that transduce the TNF signal further. In one case, the p55 receptor cytosolic domain is linked to pathways of cell death, whereas the p75 is not. Both receptors, however, result in the translocation of the NF-κB to the nucleus, where it binds to the promoter regions of a variety of genes. These gene products are often the same as those triggered by IL-1, which also results in the translocation of NF-κB to the nucleus. The difference is, however, that the cytosolic domains of the p55 TNF receptor (TNFR) are unique in their ability to activate intracellular signals leading to programmed cell death (also called apoptosis). The p55 TNFR has the so-called death domain and recruits a protein called MORT-1. Also involved in this process is a family of intracellular proteins that becomes activated; these proteins are called *TNFR-associated factors*. Presently there are six or perhaps eight TNFR-associated factors. The p55 cytosolic domains also recruit the family of intracellular proteins called TNFR-associated death domains (TRADDs). The overexpression of TRADDs results in cell death. It also leads to activation of NF-κB. TRADDs also lead to the activation of the caspase family of intracellular cysteine proteases. Although caspase-1 (also knows as the IL-1β-converting enzyme) is important for processing the precursors for proIL-1β and proIL-18, other members of this family are also part of the TNF cell death signaling pathway.

One interesting aspect of the biology of TNF in the brain is its ability to both protect neurons as well as to initiate their self-destruction. Both pathways involve the activation of NF-κB.[36] In general, the state of the cell (cell cycle) may help to explain why the activation of NF-κB can be associated with both the protection of cell death as well as apoptosis. One is reminded that the activation of NF-κB leads most often to new protein synthesis; some proteins from this process are clearly inducing cell proliferation, whereas others induce cell death.

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National Law Journal

December 8, 2003

SECTION: NEWS; Vol. 26; No. 15; Pg. S3

LENGTH: 2151 words

HEADLINE: Early-stage companies face new challenges;
'Rochester' case limited the patentability of 'reach-through' claims.

BYLINE: By Stephen R. Albainy-Jenei and Karlyn A. Schnapp
Special to The National Law Journal; Stephen R. Albainy-Jenei and Karlyn A. Schnapp are associates and patent attorneys in the Cincinnati office of Frost Brown Todd. They handle a diverse intellectual property practice in the biotechnology, pharmaceutical and chemical fields. They may be reached at mailto:salbainyjenei@fbtlaw.com and mailto:kschnapp@fbtlaw.com, respectively.

BODY:

Broad patent protection is essential to the development of biotechnology. Early-stage biotechnology companies consume large amounts of capital and resources, known as the burn rate, to develop new therapeutics even while the failure rates in the biotechnology industry are extraordinary. For illustration, in order to get just one new drug approved by the Food and Drug Administration, companies will typically screen 5,000 to 10,000 compounds, spending $800 million over the course of 14 years from initial screening to FDA approval.

In view of these tremendous commercialization costs, early-stage companies generally must obtain venture capital to survive. However, investment is only available when exclusivity to the technology rights can be obtained to balance the risk to venture capital companies. Thus, comprehensive patent protection is crucial in biotechnology for obtaining financing and is a necessary piece to corporate partnering transactions.

Therefore, early-stage companies often look to obtain patent protection for downstream inventions by way of "reach-through claims." This type of patenting attempts to capture rights of future discoveries made through the use of early, often fundamental or enabling discovery. For example, researchers may make a breakthrough discovery of a key therapeutic target, such as a gene or enzyme that can be modulated to offer a therapeutic effect. Future discoveries may then be made when the new target is used to identify previously unknown therapeutic compounds that modulate the activity of this target.

Greater upstream protection yields greater control over later developments and downstream technologies. But stricter patentability requirements that disproportionately affect the types of breakthrough inventions that are seen in biotechnology have made the maximizing of the economic value of those inventions more challenging.

In decisions such as Regents of the University of California v. Eli Lilly & Co., 119 F.3d 1559 [Fed. Cir. 1997]; Enzo Biochem Inc. v. Gen-Probe Inc., 296 F.3d 1316 [Fed. Cir. 2002]; and Fiers v. Revel, 984 F.2d 1164 [Fed. Cir. 1993], the courts have relied upon the written-description requirement to invalidate a number of biotechnology patents. This requirement, under 35 U.S.C. 112, 1, states that a patent specification must adequately describe the invention. This is deemed necessary to ensure that inventors actually have invented what is claimed.

The enablement requirement, which is separate from the written-description requirement, arises out of this same section and ensures that patent specifications adequately teach how to "make and use" the claimed invention.

This year, in University of Rochester v. G.D. Searle, 249 F. Supp. 2d 216 [W.D.N.Y. 2003], a U.S. district court found all claims of the University of Rochester's patent invalid on two separate grounds-a lack of written description and a lack of enablement for these claims-and dismissed the university's lawsuit against Pfizer, Searle, Monsanto and Pharmacia. The patent included method-of-treatment claims directed to nonsteroidal anti-inflammatory drugs that selectively inhibit one of two enzymes implicated in inflammatory pain, Cox-2 but not Cox-1, without concomitant stomach inflammation. Traditional pain relievers inhibit the activity of both enzymes, resulting in stomach irritation.

The patent involved two different types of claims that purported to cover all compounds capable of selectively inhibiting Cox-2. Although the university argued that its breakthrough discovery provided the means for identifying successful inhibitors for use as nonsteroidal anti-inflammatory agents, the court observed that it failed to describe or enable even a single compound. The specification merely included a list of possible classes of compounds that could be identified in a screening assay along with general descriptions of formulations and routes of administration. The fact that the claimed invention was a method and not merely a compound did not change the invalidity analysis.

In granting summary judgment of invalidity for failure to comply with the written-description requirement, Judge David G. Larimer stated that while the court's decision "does not mean to suggest that the inventors' significant work in this field is on par with alchemy, the fact remains that, without the compound called for in the patent, the inventors could no more be said to have possessed the complete invention claimed in the Rochester patent than the alchemists possessed a method of turning base metals into gold." Id. at 230 This was the result for a patent that the university first announced would bring in close to $1 billion in revenue.

The patent was also invalidated for failure to comply with the enablement requirement. The court held that the patent did not contain sufficient detail to enable someone to carry out the invention in showing how to select the correct compound without undue experimentation; it only provided a starting point.

Strategies in light of 'Rochester'

While Rochester is on appeal to the U.S. Court of Appeals for the Federal Circuit, it is likely that such reach-through claims will remain severely restricted, possibly hurting the value of intellectual property for many early-stage biotechnology companies. Given this trend, how can early-stage biotechnology companies develop adequate patent protection to support R&D investment?

First, the researcher should file a patent application when at least one compound that has been identified can perform the specific function claimed. If at all possible, patent filings should be delayed until there is actual reduction to practice of the claimed subject matter. In relying on Eli Lilly, Larimer held that generic formulae are normally an adequate description of the claimed genus [for nongenetic material], but the Cox-2 patent specification stated only that suitable compounds might be found from among an array of organic and inorganic materials. Thus, if the researcher can identify a single compound for practicing the claimed method, it should then be possible to construct both genus and species claims for a series of related chemical compounds that would satisfy the written-description requirement.

Second, if a compound cannot be identified and a patent filing cannot be delayed, then the researcher should file an application with method-of-treatment claims when support can be derived from a structural and functional characterization of claimed compounds, preferably using data from relevant models. Generally, a description of a theoretical compound that includes only its desired biological function or activity does not satisfy the written-description requirement because it does not show that the inventors were actually in possession of an actual compound that could be used to practice the invention. But, relying on Enzo, Larimer stated that a description of functional characteristics can meet the written-description requirement, in accordance with Patent and Trademark Office guidelines, if it is "sufficiently detailed and provides relevant identifying characteristics," including "functional characteristics when coupled with a known or disclosed correlation between function and structure." Id. at 226.

While Larimer held that no such correlation was disclosed in the Cox-2 patent, he did open the door for such claims. Accordingly, the specification must make it sufficiently clear that the methods in the specification can be performed or that the product claimed is produced by the process. Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, 1, "Written Description" Requirement, 66 Fed. Reg. 1099 [Jan. 5, 2001].

Third, if the researcher is faced with the dilemma in which there are currently no known compounds to describe, yet there is an immediate need to file a patent application, claims can be directed to a screening assay useful for identifying compounds important for targeting the desired biochemical pathway. For instance, borrowing language from the Cox-2 patent, U.S. Pat. No. 6,048,850, a sample claim might read: "A biological screening assay for testing

compounds that selectively inhibit PGHS-2 activity, said assay comprising administering a non-steroidal compound that selectively inhibits activity of the PGHS-2 gene product and ascertaining the compounds that had a positive response to said biological screening assay by measuring the conversion of arachidonic acid to its prostaglandin metabolite; so that the compounds that inhibit PGHS-2 and not PGHS-1 activity are identified."

However, these claims may be more difficult to assert since it is often difficult to discover a company's drug-discovery methods. Alternatively, an option may be to draft claims directed to the target for drug screening by defining functional domains, preferably by structure, that can be used in the screening assay. For gene claims, one can describe homologs from other species to define structurally related classes of molecules, preferably identifying any conserved domains. It is also valuable to include any known fragments and variants.

Claims can also be directed to in vitro methods of using the target. For example, method claims might include, "A method of optimizing a model for predicting a pharmacokinetic property of a compound in a mammalian biochemical pathway of interest, the method comprising...."

Fourth, the Rochester decision appears to shift greater rewards further toward downstream research and product development. As Judge Randall R. Rader noted while concurring in Moba B.V. v. Diamond Automation Inc., 325 F.3d 1306, 1325 [Fed. Cir. 2003], "the Lilly rule can also have the unintended consequence of pricing non-corporate inventors out of the inventive market for biotechnology." Therefore, it may be necessary for early-stage companies to undertake co-development agreements with partners able to perform the necessary further drug screening in order to distinguish the essential class of compounds.

Creative claiming

The consequence for many of these approaches is that the patent practitioner must be creative when claiming the applicant's invention and must formulate as many different types of commercially useful claims as possible. Beyond the strategies discussed above, another tactic in view of the Cox-2 case would be to file an initial patent application on a narrow embodiment that is adequately described and enabled within the metes and bounds of 35 U.S.C. 112. Then the practitioner should file subsequent patent applications once other lead compounds have been identified, thereby creating a series of related patents. With this type of strategy, a series of narrowly drafted and claimed patent applications would be pursued.

This type of strategy may be successful in overcoming the enablement and written-description issues associated with the Cox-2 patent. However, the overall cost in fees for drafting and prosecuting such a large number of applications would pose a detriment to many universities and small biotech start-ups.

It is clear from the court's decision that for those patent applications that are directed to biochemical pathways, broadly constructed reach-through claims will have a difficult time meeting the 35 U.S.C. 112 requirements. The immediate implications are that more money will need to be spent in R&D at the front end, directed primarily to developing lead compounds. In addition, the overall cost in legal fees for drafting and prosecuting more carefully crafted, fully detailed biotechnology applications will only increase for complex inventions.

While big pharmaceutical companies will have the money to spend in such endeavors, it will be the universities and the small biotech start-ups that will most certainly be affected since these institutions historically do not have the resources, both financial and in personnel, to overcome this new set of obstacles in trying to obtain patent protection for their scientific contributions in an ever-changing landscape.

It is possible that ongoing developments in high-throughput screening and structure-based design will change the level of actual results required in the written description. What is unpredictable or requires undue experimentation today will be routine tomorrow.

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were moved, as one, from trial to trial, rather than from session to session. Moving the platform from trial to trial makes it impossible to find by reference to the room cues alone, which will have two consequences. First, group 'trial' cannot show the within-session improvement in performance that would be expected for group 'session'. Second, the tendency to search in the region in which the platform had been located previously at the start of each session should be less for group 'trial' than for group 'session'. The improvement in performance in the first trial of successive sessions should thus be more marked for group 'trial' than for group 'session'. In other words, if our analysis of the performance of the hippocampally damaged rats is correct, moving the platform from trial to trial should have exactly the same effect as a hippocampal lesion.

In support of the above prediction, the results for group 'trial' and group 'session' (Fig. 5) show a striking similarity to those for, respectively, the hippocampally damaged and control groups (Fig. 3). Group 'trial' and the hippocampally damaged rats took less time to find the platform in the first trial of most sessions than did their respective control groups. Furthermore, group 'trial' and the hippocampally damaged rats both failed to show the within-session improvement in performance that was characteristic of the other two groups. An ANOVA using individual mean escape latencies for the 11 sessions combined showed that the latency to escape from the pool was significantly longer in trial 1 than trial 4 for group 'session' ($F = 130.44$, d.f. = 1/14), but not group 'trial' ($F = 3.25$, d.f. = 1/14). There was also a significant difference between the escape latencies for the two groups in trial 1 and trial 4 (F values > 11.24, d.f. = 1/28).

The gradual improvement in performance in the first trial of each session for normal rats in experiment 1, and for group 'session' in experiment 2, shows that more reliance was placed on the heading-vector strategy, and less on the cognitive-map strategy, as training progressed. According to theories of learning this change would occur because stimuli, or in this case navigational strategies, must compete for the control they acquire over behaviour[8]. At first, animals may be predisposed to adopt a cognitive-map strategy, based on cues outside the pool. However, because the platform can be found more reliably by reference to a landmark-based heading vector than by a cognitive map, the theories predict that progressively more reliance will be placed on the heading vector. As a result of this switch in strategy, rats should eventually find the platform swiftly at the start of a session, and when the platform and landmark occupy a new position.

Both experiments show that normal rats escaped from the pool by means of two different navigational strategies when the platform remained in the same place for all the trials of a session. One strategy was based entirely on cues that surrounded the pool, and could have involved information about the geometric relationship between the platform and a set of these cues. In other words, rats used a cognitive map to define the position of the platform. The second strategy involved the landmark within the pool, and required rats to learn the direction and distance of the platform from the landmark. That is, rats may also have used a heading vector to define the position of the platform. The results from experiment 1 show that damage to the hippocampus disrupts the first of these strategies, but not the second. □

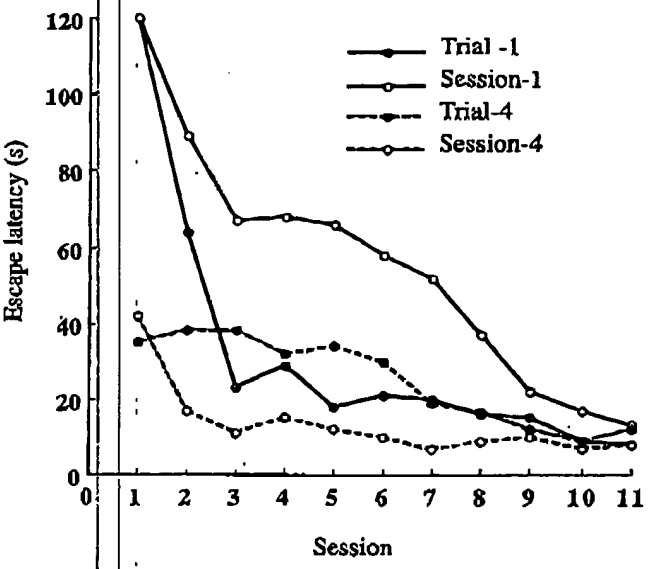

Figure 6 Mean latency of escape in the first and fourth trials of each of the 11 sessions of training, for a group trained with the platform in the same place throughout each session (Session-1 and Session-4) and for a group trained with the platform moved after each trial (Trial-1 and Trial-4).

Methods

The Morris pool was in a well-lit rectangular room with brightly coloured posters on the wall. The lowest point of the spherical black landmark was 2 cm below the surface of the water. For every training trial the midpoint of the 20-cm gap between the sides of the platform and the landmark was located at the midpoint of the radii that pointed towards the eight main points of the compass (Fig. 1). A different release point on the edge of the pool was randomly selected for each training trial. Rats remained on the platform for 30 s once they had climbed onto it. Further details of the method can be found in ref. 4.

Received 29 May; accepted 28 August 1998.

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Acknowledgements. We thank R. Honey, E. S. Redhead and R. G. M. Morris for advice and discussions. This work was supported by a DIISRC studentship, and by a grant from the UK Biotechnology and Biological Sciences Research Council.

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The anti-inflammatory agents aspirin and salicylate inhibit the activity of IκB kinase-β

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NF-κB comprises a family of cellular transcription factors that are involved in the inducible expression of a variety of cellular genes that regulate the inflammatory response[1,2]. NF-κB is sequestered in the cytoplasm by inhibitory proteins, IκB, which are phosphorylated by a cellular kinase complex known as IKK. IKK is made up of two kinases, IKK-α and IKK-β, which phosphorylate IκB, leading to its degradation and translocation of NF-κB to the nucleus[3–9]. IKK kinase activity is stimulated when cells are

exposed to the cytokine TNF-α or by overexpression of the cellular kinases MEKK1 and NIK[10,11]. Here we demonstrate that the anti-inflammatory agents aspirin and sodium salicylate specifically inhibit IKK-β activity *in vitro* and *in vivo*. The mechanism of aspirin and sodium salicylate inhibition is due to binding of these agents to IKK-β to reduce ATP binding. Our results indicate that the anti-inflammatory properties of aspirin and salicylate are mediated in part by their specific inhibition of IKK-β, thereby preventing activation by NF-κB of genes involved in the pathogenesis of the inflammatory response.

Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin, sodium salicylate, and indomethacin exert their anti-inflammatory effects at least in part by inhibition of the enzyme cyclooxygenase[12,13]. Aspirin and sodium salicylate can also inhibit the NF-κB pathway which is involved in the pathogenesis of the inflammatory response[14,15]. We have looked for cellular targets that regulate NF-κB and could potentially be inhibited by these anti-inflammatory agents. First we assayed the effect of aspirin and sodium salicylate on inhibiting TNF-α and NIK activation of NF-κB-mediated gene expression[10,16]. At concentrations (1–5 mM) measured in the serum of patients treated with aspirin and sodium salicylate for chronic inflammatory diseases[17], these agents strongly inhibited NIK- and TNFα-activated gene expression from the two NF-κB sites in a human immunodeficiency virus (HIV-1) long terminal repeat (LTR) chloramphenicol acetyltransferase (CAT) reporter plasmid that was transfected into Jurkat cells (Fig. 1a). Aspirin and salicylate also strongly inhibited NF-κB activation by the human T-lymphotropic virus (HTLV-1) transactivator Tax and the MAP3 kinase MEKK1 (ref. 11) (Fig. 1b). Neither NIK nor TNF-α activated gene expression from an HIV-1 LTR reporter construct containing mutated NF-κB sites (data not shown). In contrast to the potent inhibition of aspirin and salicylate on NF-κB activation, neither dexamethasone[18,19] nor the cyclooxygenase inhibitor indomethacin[19] prevented NF-κB activation by TNF-α, NIK, Tax or MEKK1 (Fig. 1a, b), indicating that aspirin and sodium salicylate inhibition of the NF-κB pathway might not strictly depend on inhibition of prostaglandin synthesis. Finally, we tested whether either aspirin or salicylate could inhibit activation of gene expression by other kinase pathways. The adenovirus early-region-3 promoter, whose gene expression is activated by protein kinase A phosphorylation of CREB, was not inhibited by aspirin and salicylate treatment in the presence of forskolin[20] (Fig. 1c). This indicated that aspirin targets only a subset of kinase pathways that activate gene expression.

One of the critical steps in the activation of the NF-κB pathway is phosphorylation by IKK of IκB, which leads to its degradation[5–9]. Kinase activity was assayed with a fusion GST–IκBα substrate (where GST is glutathione *S*-transferase) using IKK immunoprecipitated from cells treated with TNF-α in both the presence or absence of anti-inflammatory agents. Aspirin and sodium salicylate, but not dexamethasone or indomethacin, resulted in a 75% inhibition of endogenous IKK kinase activity (Fig. 1d, top). IKK did not phosphorylate a GST–IκBα substrate mutated at serine residues 32 and 36, which are required for IκBα phosphorylation and subsequent degradation (data not shown). Although treatment of HeLa cells with aspirin, sodium salicylate or indomethacin decreased prostaglandin levels, only aspirin and sodium salicylate prevented

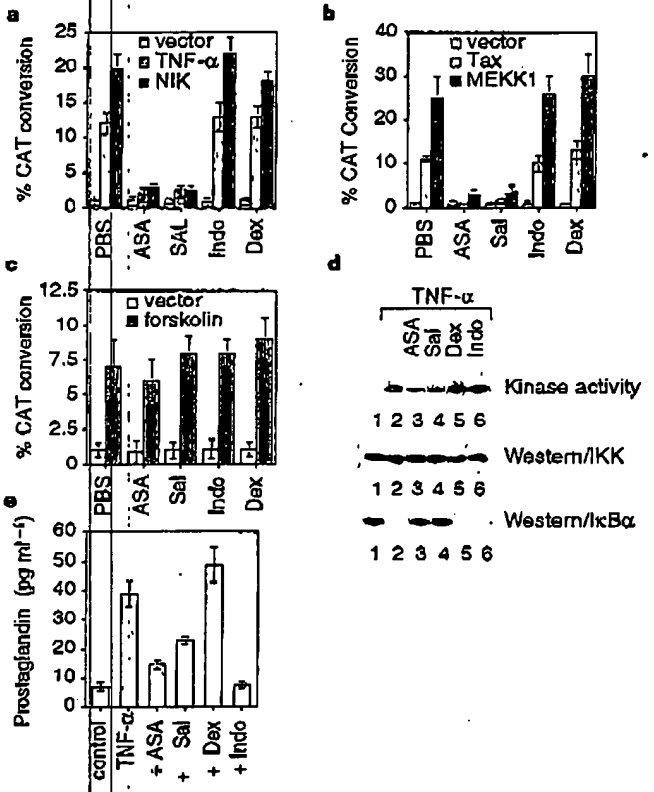

Figure 1 Aspirin inhibits NF-κB activation and IKK kinase activity. Jurkat cells were transfected with an HIV-1 LTR CAT construct and treated with **a**, TNF-α (20 ng ml^{-1}) or an NIK expression vector; **b**, *tax* or an MEKK1 expression vector; or **c**, an E3CAT reporter in the presence of forskolin (25 μM). Cells were treated with either phosphate-buffered saline or agent (ASA, aspirin; SAL, sodium salicylate; Indo, indomethacin; Dex, dexamethasone) and the fold increase in gene expression in the presence of different activators was calculated by phosphorimager quantification. **d**, TNF-α-treated HeLa cell extracts were assayed for endogenous IKK kinase activity (top) and western blot analysis for either IKK-α (middle) or IκBα (bottom). **e**, Tissue culture medium in **d** was assayed for secreted prostaglandins.

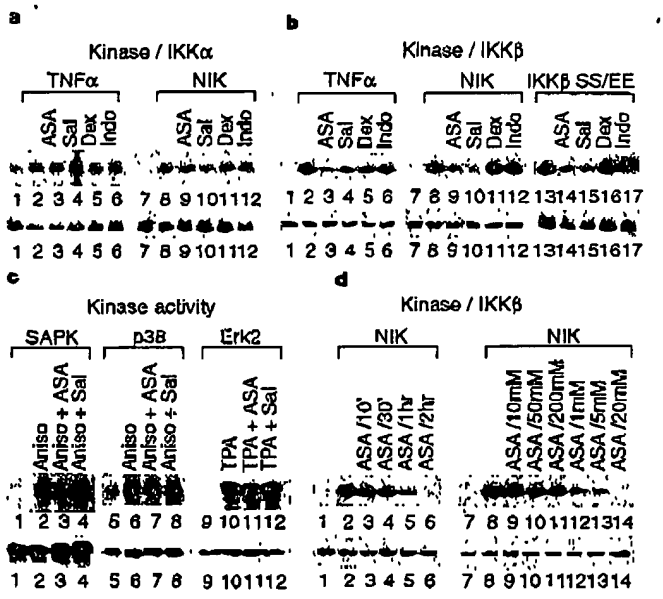

Figure 2 Aspirin specifically inhibits IKK-β kinase activity. Epitope-tagged expression vectors for **a**, IKK-α; **b**, IKK-β; **c**, SAPK, p38 and Erk2; or **d**, IKK-β were transfected into COS cells and treated with either TNF-α or NIK, while SAPK and p38 were treated with anisomycin and Erk2 with phorbol esters following 2-h treatment with the indicated anti-inflammatory agents. Immunoprecipitated kinases were assayed with substrates including: **a**, **b**, **d**, GST-IκBα (aa 1–54); or **c**, GST-Jun (aa 1–169) (lanes 1–4), GST-ATF2 (aa 1–254) (lanes 5–8) or myelin basic protein (lanes 9–12). **d**, Aspirin (5 mM) was added to cells transfected with IKK-β for various times as indicated (lanes 3–6), or different concentrations of aspirin were added for 2 h before collecting cells (lanes 9–14) and IKK-β kinase activity was determined. Western blot analysis of each of these immunoprecipitated kinases is shown in the lower panels.

TNF-α induction of endogenous IKK activity (Fig. 1d, e). Thus, a reduction in prostaglandin synthesis alone was not sufficient to inhibit IKK activity.

To determine whether the activity of either IKK-α or IKK-β could be specifically inhibited by treatment of cells with aspirin or salicylate, expression vectors containing epitope-tagged IKK-α or IKK-β were transfected into COS cells in the presence of either NIK or TNF-α. Both IKK-α and IKK-β activity was increased by treatment of cells with TNF-α and NIK (Fig. 2a, b, lanes 1 and 2). Aspirin and salicylate inhibited IKK-β but not IKK-α kinase activity (Fig. 2a, b, lanes 3 and 4). A constitutively active IKK-β kinase (amino-acid residue mutations S177E and S181E, SS → EE)[7] was also inhibited by aspirin and sodium salicylate treatment (Fig. 2b, lanes 14 and 15), suggesting that these agents may inhibit IKK-β rather than upstream regulatory kinases. Neither aspirin nor sodium salicylate inhibited the activity of the stress-activated protein kinases SAPK, p38 and Erk2 (refs 20–24) (Fig. 2c, lanes 1–12). Finally, we determined the time course and dose dependence *in vivo* of aspirin treatment of cells transfected with an IKK-β expression vector. NIK activated IKK-β kinase activity 40-fold and 50% inhibition of IKK-β kinase activity was seen with aspirin treatment for 30 min (Fig. 2d, lanes 2 and 4) and with an aspirin concentration of 50 μM (Fig. 2d, lane 10). These results supported the conclusion that aspirin and salicylate selectively inhibit IKK-β activity.

Next we investigated whether aspirin could inhibit IKK-α, IKK-β or IKK-β(SS → EE) kinase activity following immunoprecipitation and *in vitro* incubation with either aspirin (Fig. 3a, lanes 1–12) or indomethacin (Fig. 3a, lanes 13–18) for the times indicated. Aspirin treatment did not alter IKK-α activity when produced alone or in the presence of NIK (Fig. 3a, lanes 1–6). In contrast, the kinase activity of both wild-type IKK-β and the constitutively active IKK-β(SS → EE) were both decreased by more than 90% when treated *in vitro* with aspirin (Fig. 3a, lanes 7–12). *In vitro* treatment with indomethacin did not decrease the kinase activity of either the wild-type or the constitutively active IKK-β(SS → EE) (Fig. 3a, lanes 13–18). Consistent with our *in vivo* results, *in vitro* treatment with aspirin did not alter the kinase activity of immunoprecipitated SAPK, p38 or Erk2 (Fig. 3b, lanes 1–12). Aspirin also inhibited the *in vitro* kinase activity of baculovirus-expressed IKK-β (Fig. 3c, lanes 3–6) but not IKK-α (Fig. 3c, lanes 1–3). The half-maximal inhibitory concentration (IC_{50}) of aspirin required to inhibit prostaglandin synthesis has been reported to range from 50–100 μM[25,26]. The IC_{50} for *in vivo* aspirin treatment required to inhibit endogenous IKK kinase activity was 80 μM (Fig. 4a). Aspirin

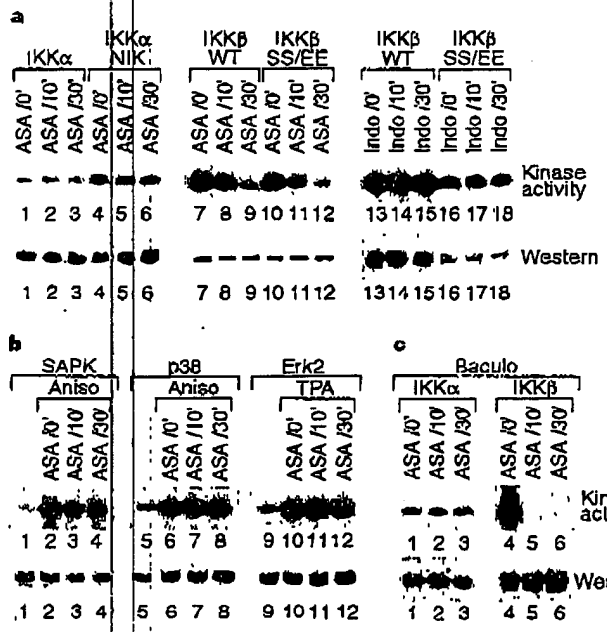

Figure 3 Aspirin inhibits IKK-β kinase activity *in vitro*. COS cells were transfected with: **a**, epitope-tagged IKK-α or IKK-β, or **b**, SAPK, p38, or Erk2 cDNAs treated with either anisomycin or phorbol esters. Kinases were immunoprecipitated and incubated *in vitro* for 0, 10 or 30 min with either 1 mM aspirin or 25 μM indomethacin, followed by kinase assays. **c**, Baculovirus-produced IKK-α (lanes 1–3) or IKK-β (lanes 4–6) proteins were also incubated *in vitro* with 1 mM aspirin for either 10 or 30 min and assayed for kinase activity. Lower panels, western blot analysis of each immunoprecipitate. WT, wild type; SE/EE, mutants.

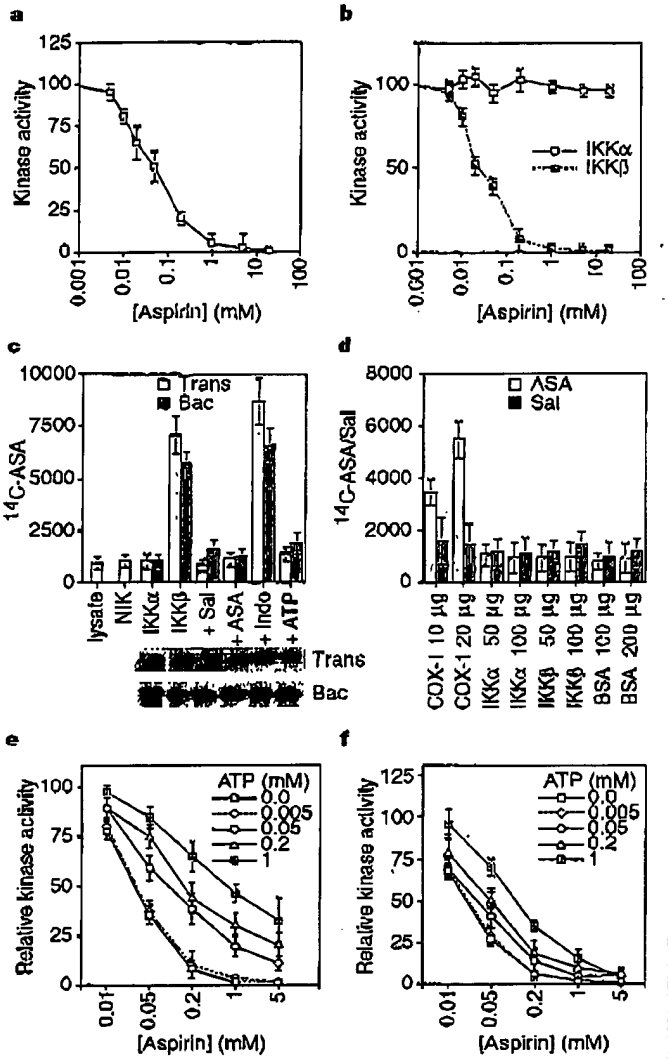

Figure 4 Aspirin and salicylate compete with ATP for binding to IKK-β. **a**, HeLa cells were treated with various concentrations of aspirin *in vivo* for 2 h before TNF-α treatment and assay of endogenous IKK activity. **b**, Baculovirus-produced IKK-α and IKK-β proteins were incubated *in vitro* with various concentrations of aspirin and assayed for kinase activity. **c**, NIK, IKK-α or IKK-β proteins produced following either transfection (Trans) or baculovirus (Bac) expression were immunoprecipitated and incubated with ^{14}C-aspirin in the presence of a 500-fold molar excess of unlabelled competitors and binding was quantified; western-blot analysis of the immunoprecipitated IKK proteins is shown in the lower panel. **d**, Purified IKK-α, IKK-β or COX-1 proteins were incubated with either ^{14}C-aspirin or ^{14}C-salicylic acid, precipitated with 20% TCA and quantified by β counting. **e**, **f**, Purified IKK-β was incubated with **e**, different concentrations of aspirin and ATP added together, or **f**, preincubated with aspirin for 30 min before addition of ATP and assayed for kinase activity.

also inhibited the *in vitro* kinase activity of baculovirus-expressed IKK-β but not IKK-α at 30–50 μM (Fig. 4b). Thus, the IC_{50} for *in vitro* aspirin inhibition of IKK-β correlated with the IC_{50} for *in vivo* aspirin inhibition of endogenous IKK.

We next assayed the ability of aspirin and salicylate to bind to IKK-α or IKK-β isolated from either transfected cells or purified following baculovirus expression. Sevenfold more ^{14}C-aspirin bound to IKK-β than to either IKK-α or NIK (Fig. 4c). Moreover, the binding of ^{14}C-aspirin or [7-^{14}C]salicylic acid (data not shown) to IKK-β was competed by a 500-fold molar excess of either unlabelled salicylate or aspirin, but not indomethacin (Fig. 4c). A 500-fold molar excess of ATP was also able to compete with the binding of ^{14}C-aspirin to IKK-β, indicating that ATP and aspirin competed for binding to IKK-β (Fig. 4c). To determine whether aspirin or salicylate was covalently bound to IKK-β, we used trichloroacetic acid (TCA) to precipitate recombinant IKK-α and IKK-β bound to either ^{14}C-salicylate or ^{14}C-aspirin (Fig. 4d). TCA precipitation did not reveal significant amounts of ^{14}C-aspirin or ^{14}C-salicylate remaining bound to precipitated IKK-β. In contrast, the cyclooxygenase COX-1, which binds covalently to aspirin but not salicylate, bound to ^{14}C-aspirin but not ^{14}C-salicylate (Fig. 4d)[25,26]. These results indicate that aspirin and salicylate do not bind covalently to IKK-β.

To confirm that aspirin and salicylate compete with ATP for binding to IKK-β, we preincubated increasing concentrations of ATP and aspirin with purified IKK-β before assaying for kinase activity. The IC_{50} of aspirin required to inhibit IKK-β shifted with increasing ATP concentration, indicating that aspirin was a competitive inhibitor of ATP binding to IKK-β (Fig. 4e). When aspirin was preincubated with IKK-β for 30 min before addition of ATP, the IC_{50} of aspirin did not markedly shift with increasing ATP concentration (Fig. 4f), indicating that the binding of aspirin to IKK-β was either irreversible or very slowly reversible, although this binding is not covalent. As NF-κB is a key cellular regulator of the inflammatory response, inhibition of the nuclear translocation of NF-κB should suppress the host inflammatory response. Our identification of IKK-β as a target for sodium salicylate and aspirin should help in the design of aspirin-like agents to inhibit the inflammatory response even more effectively. □

Methods

Cell culture and transfections. COS and HeLa cells were transfected with Fugene 6 (Boehringer–Mannheim); Jurkat cells were transfected with DEAE–dextran. Cells were collected 24 h post-transfection in the absence or presence of aspirin (5 mM), sodium salicylate (5 mM), dexamethasone (10 μM) or indomethacin (25 μM). The HIV1-LTR-CAT and E3-CAT reporter constructs[11,20], and the epitope-tagged IKK-α (HA), IKK-β (Flag), NIK (c-Myc), Tax, MEKK1, p38 (HA), SAPK (Myc), Erk2 (Myc) have been described[11,21–24]. TNF-α (20 ng ml^{-1}) was added to cells for 10 min before collection to stimulate IKK kinase activity and for 20 h post-transfection for assay of NFκB-mediated gene expression. Cells transfected with SAPK and p38 cDNAs were treated with anisomycin (10 μg ml^{-1}) for 30 min before collection; cells transfected with the Erk2 cDNA were pretreated with TPA (12-*O*-tetradecanoylphorbol-13-acetate; 50 ng ml^{-1}) for 30 min[24] to activate these kinases. Both aspirin (acetyl salicylic acid) and sodium salicylate (Sigma) were dissolved in 0.05 M Tris-HCl to prepare 1.0 M stock solutions; dexamethasone and forskolin (Sigma) were prepared as described[18]. Supernatants from cells were applied to a C18 minicolumn and assayed for prostaglandin using an ELISA kit (Amersham).

Kinase assays. Lysates (200 μg protein) were prepared from transfected cells and incubated with antibody (anti-Flag (M2), anti-HA (12CA5), or anti-Myc) at 4 °C for 1 h and 20 μl protein A–agarose was added for 1 h. After extensively washing the immunoprecipitates, kinase assays were done as described[11]. For *in vitro* kinase assay, aspirin was added into the washed immunoprecipitates for 30 min at 4 °C before the kinase reaction. Mixtures were subjected to SDS–PAGE and autoradiography and quantified by phosphorimager analysis.

Calculation of the IC_{50} of aspirin. To assay endogenous IKK activity, cell lysates (200 μg protein) were immunoprecipitated with a rabbit polyclonal antibody directed against IKK-α (Santa Cruz) that immunoprecipitates the IKK-α/IKK-β heterodimer, followed by assay of kinase activity. Polyhistidine and Flag-tagged IKK-α and IKK-β proteins were produced by baculovirus expression and purified using nickel-agarose chromatography. Purified proteins (500 μg) were immunoprecipitated using 12CA5 monoclonal antibody, divided into ten equal fractions and each was treated with a different concentration of aspirin or sodium salicylate for 30 min on ice. Kinase activity was then assayed and quantified by phosphorimager; aspirin inhibition of kinase activity was calculated and plotted against aspirin concentration.

IKK binding to ^{14}C-salicylate and ^{14}C-aspirin binding. Proteins (200 μg) isolated from baculovirus-expressed and purified IKK-α and IKK-β proteins or cells transfected with IKK-α or IKK-β cDNAs were immunoprecipitated with epitope-specific monoclonal antibodies and then incubated with 500 μl binding buffer containing 100 mM NaCl, 50 mM Tris, pH 7.5, 10 mg ml^{-1} BSA, protease inhibitors, and 2 μCi of either acetyl salicylic ^{14}C-carboxylic acid or [7-^{14}C]salicyclic acid (40–60 mCi mmol^{-1}). A 500-fold molar excess (36 mM) of aspirin, sodium salicylate, indomethacin or ATP was added to each immunoprecipitate and incubated at 4 °C for 30 min. Immunoprecipitates were then washed extensively with binding buffer and the amount of ^{14}C-salicylate or ^{14}C-aspirin bound was quantified by β-counting. Immunoprecipitates were also incubated with 20% TCA, precipitates were isolated by centrifugation and dissolved in 1 M NaOH. The amount of ^{14}C-aspirin and ^{14}C-salicylate in the protein precipitates was quantified by β counting. Either 10 or 20 μg COX-1 protein (from Cayman Chemical) was used in binding reactions with IKK proteins.

Received 15 August; accepted 17 September 1998.

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Acknowledgements. We thank F. Mercurio for IKK-α and IKK-β cDNAs, M. Cobb and E. Ross for discussion, and S. Johnson and E. Fasternack respectively for preparing the manuscript and figures. This work was supported by grants from the NIH, the Council for Tobacco Research and the Robert Welch Foundation.

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Foye's Principles of Medicinal Chemistry

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Designer: Armen Kojoyian
Compositor: Graphic World
Printer: Quebecor World-Dubuque

351 West Camden Street
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530 Walnut Street
Philadelphia, PA 19106

Printed in the United States of America

First Edition, 1974

Library of Congress Cataloging-in-Publication Data
Williams, David A., 1938-
Foye's principles of medicinal chemistry/David A. Williams, Thomas L. Lemke.—5th ed.
p. cm.
Includes index.
ISBN 0-683-30737-1
1. Pharmaceutical chemistry. I. Title: Principles of medicinal chemistry. II. Lemke, Thomas L. III. Title.

RS403 .P75 2002
616.07'56—dc21 2001050327

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leaves, flowers, sap etc.). Each method of administration or part of the plant may involve different chemical compounds to produce the desired outcome. One can readily see that isolation of active constituents from plants that may be useful as medicinals is not a simple process and a number of variables are involved that may influence the amount of active compound or compounds that may influence the pharmacologic activity of the extract.

Random Screening of Synthetic Organic Compounds

This approach to discovering new chemical structures for a particular biological action began in the 1930s after the discovery of the sulfonamide class of antibacterials. Thousands of compounds and their synthetic intermediates were assayed in search of new structures that possessed antibacterial activity. All compounds available to the investigator (natural products, synthetic compounds), regardless of structure, were tested in the assays available at the time. This approach was also applied in the 1960s and 1970s in an effort to find agents that were effective against cancer. Some groups did not limit themselves to a particular biological activity, but tested compounds in a wide variety of assays. This approach was a precursor to what is now referred to as high throughput screening assays. This involves the bioassay of thousands of compounds in hundreds to thousands of bioassays simultaneously. This only became possible with the advent of computer controlled robotic systems for the assays and combinatorial chemistry techniques. These will be discussed further below.

What is crucial for random screening to be successful is a good bioassay system for the pharmacologic action of interest. Unfortunately, this means of lead discovery is very inefficient because no rational approach is taken to what compounds are to be tested to find new lead structures. Random screening eventually gave way to dedicated screening and rational design techniques.

Targeted Dedicated Screening and Rational Drug Design

This approach is more or less random in nature and involves greater knowledge of the therapeutic targets and some actual design based on physicochemical properties. Testing is usually with one or two models (e.g., specific receptor systems or enzymes) based on the therapeutic target. The design aspect often involves molecular modeling and the use of quantitative structure-activity relationships (QSAR) to better define the physicochemical properties that are crucial for biological activity. The drawback of these approaches is that they are better for developing a lead compound rather than discovery of the lead compound.

New Drug Discovery via Drug Metabolism Studies

New compounds have been "discovered" by investigating the metabolism of compounds that already are clinical candidates or, in rare instances, compounds that are already on the market. Metabolites of known compounds are isolated and then assayed for biological effects either on the same target system or a broader screen of several other target systems. The latter will be more useful if the metabolite being studied is a chemical structure that has been radically altered from the parent molecule through some unusual rearrangement reaction. More often the metabolite is not radically different from the parent molecule and therefore would be expected to have similar pharmacologic effects. The advantage is that a metabolite may possess better pharmacokinetic properties such as a longer duration of action, better absorption orally, or less toxicity with fewer side effects (e.g., terfenadine and its antihistaminic metabolite, fexofenadine). The sulfonamide antibacterial agents were discovered in this way. The azo dye prontosil was found to have antibacterial action in vitro only. It was soon discovered that this compound required reduction of the diazo group to produce 4-aminobenzene sulfonamide (Fig. 2.27) which was found to act as an antagonist to p-aminobenzoic acid, a crucial component in microbial metabolism.

New Drug Discovery via Observation of Side Effects

An astute clinician or pharmacologist may detect a side effect in a patient or animal model that could lead, upon further development, to a new therapeutic use for a particular chemical structure. Further development may even lead to an entirely new chemical class. This discovery of new lead compounds has occurred several times and will be discussed below.

One of the more interesting cases of drug development is that of the phenothiazine antipsychotics. These compounds can be traced back to the first histamine H_1-receptor antagonists developed in the 1930s. Bovet in 1937 (21) was the first to recognize that it should be possible to antagonize the effects of histamine and thereby treat allergic reactions. He tested compounds that were known to act on the autonomic nervous system and eventually discovered that benzodioxanes (Fig. 2.28) were capable of significant antagonism of the effects of histamine. In attempts to improve the antihist-

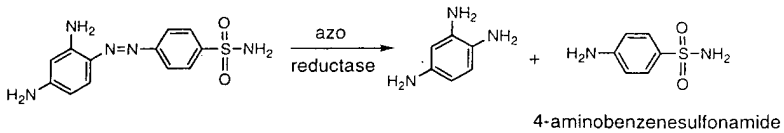

Fig. 2.27. Metabolic conversion of prontosil to 4-aminobenzenesulfonamide.

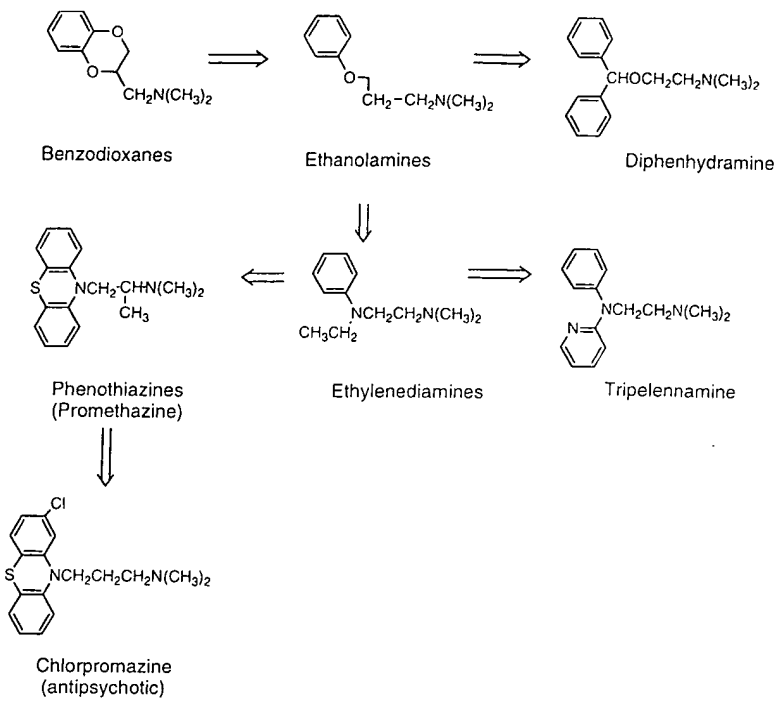

Fig. 2.28. Develpoment of phenothiazine-type antipsychotic drugs.

aminic action of the benzodioxanes it was discovered that ethanolamines also provided significant antihistaminic activity. Further development of this class ended up going in two directions. One approach led to the development of the diphenhydramine class of antihistamines and is represented by the first clinically useful H_1-receptor antagonist developed in the United States, diphenhydramine (Fig. 2.28). The other approach led to the ethylenediamine class represented by tripelennamine (Fig. 2.28).

Incorporation of the aromatic rings of the ethylenediamines into the tricyclic phenothiazine structure produced compounds (e.g., promethazine) with good antihistaminic action and relatively strong sedative properties. At first these compounds were found to not only be useful as antihistamines, but their very strong sedative properties lead to their use as potentiating agents for anesthesia (22). Further development to increase the sedative properties of the phenothiazines resulted in the development of chlorpromazine in 1950 (23).

Chlorpromazine was found to produce a tendency for sleep, but unlike the prior phenothiazines it also produced a disinterest in surroundings in patients and, with patients suffering psychiatric disorders, an ameliorative effect on the psychosis as well as relief of anxiety and agitation. These observations suggested that chlorpromazine had potential for the treatment of psychiatric disorders. Thus, what started out as attempts to improve antihistaminic activity, ultimately resulted in an entirely new class of chemical entity useful in an unrelated disorder (24).

Another example of how new chemical entities can be derived from compounds with unrelated biological effects is that of the development of the K^+ channel agonist diazoxide (Fig. 2.29). This compound was developed as the result of the observation that the thiazide diuretics such as chlorothiazide not only had a diuretic component due to inhibition of sodium absorption in the distal convoluted tubule but also a direct effect on the renal vasculature. Structural modification to enhance this direct effect led to the development of diazoxide and related K^+ channel agonists for the treatment of hypertension.

REFINEMENT OF THE LEAD STRUCTURE

Determination of the Pharmacophore

Once a lead compound has been discovered for a particular therapeutic use, the next step is to determine the pharmacophore for this compound. The pharmacophore of a drug molecule is that portion of the molecule containing the essential organic functional groups that directly interact with the receptor active site and therefore confers upon the molecule the biologic activity of interest. Since drug receptor interactions are very specific, the pharmacophore may constitute a small portion of the molecule. It has been found on several occasions that what seem to be very complex molecules can often be reduced to simpler structures with retention of the desired biological action. A well known example of this is the narcotic analgesic morphine. Morphine is a tetracyclic compound with five chiral centers. Not only would simplification of the structure possibly provide molecules with fewer side effects, but a reduction in the number of chiral centers would also greatly simplify the synthesis of morphine derivatives and thereby decrease cost. Figure 2.30 shows